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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12Q 1/68, C12N 15/11, A61K 48/00 — A1

(11) International Publication Number: WO 95/19448

(43) International Publication Date: 20 July 1995 (20.07.95)

(21) International Application Number: PCT/US95/00657

(22) International Filing Date: 13 January 1995 (13.01.95)

(30) Priority Data:
08/181,664 14 January 1994 (14.01.94) US

(71) Applicant: THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE [US/US]; 720 Rutland Avenue, Baltimore, MD 21205 (US).

(72) Inventor: SIDRANSKY, David; 3007 Northbrook Road, Baltimore, MD 21209 (US).

(74) Agents: HAILE, Lisa, A. et al.; Spensley Horn Jubas & Lubitz, 5th floor, 1880 Century Park East, Los Angeles, CA 90067 (US).

(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LT, LU, LV, MD, MG, MN, MW, MX, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ).

Published
With international search report.

(54) Title: NUCLEIC ACID MUTATION DETECTION IN HISTOLOGIC TISSUE

(57) Abstract

Methods are provided for detection of target neoplastic nucleic acids in a tissues specimen, including a tumor margin or lymph node, and reagents therefor, wherein the nucleic acids are preferably mutant tumor suppressor genes or proto oncogenes. Methods for treatment of cell proliferative diseases utilizing ribozymes or antisense oligonucleotides specific for the target mutant nucleic acids and/or replacement wild type genes are also disclosed.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method of detecting a neoplastic nucleic acid having a mutant nucleotide sequence present in a histopathologic tissue sample external to a primary neoplasm, such as a tumor margin specimen, comprising isolating the nucleic acid present in the specimen and detecting the presence of the neoplastic target nucleic acid wherein the presence of the nucleic acid sequence is known to be associated with neoplasia, such as, neoplasia of the head or neck.

The term "neoplastic" nucleic acid refers to a nucleic acid sequence which directly or indirectly is associated with or causes a neoplasm. As used herein the term "tumor margin" refers to the tissue surrounding a discernible tumor. In the case of surgical removal of a solid tumor, the tumor margin is the tissue cut away with the discernible tumor that usually appears to be normal to the naked eye. More particularly, as used herein, "margin" refers to the edge, border or boundary of a tumor. The margin generally extends from about 1mm to about 4mm from the primary tumor but can be greater depending upon the size of the primary solid tumor. The term "regional lymph node" refers to lymphoid tissue forming lymphoid organs or nodes which are in close proximity to the primary tumor. For example, regional lymph nodes in the case of head and neck carcinomas include cervical lymph nodes, prelaryngeal lymph nodes, pulmonary juxtaesophageal lymph nodes and submandibular lymph nodes. Regional lymph nodes for mammary tissue carcinomas include the axillary and intercostal nodes. The term "external to a primary neoplasm" means that the specimen is taken from a site other than directly from the primary neoplasm itself.

In its broadest sense, the present invention allows the detection of any neoplastic target nucleic acid sequence of diagnostic or therapeutic relevance, where the target nucleic acid sequence is present in a tissue sample such as that heretofore subjected to histopathologic examination using techniques of light microscopy, such as the margins of a primary tumor or a regional lymph node. Thus, the target nucleotide sequence may be, for example, a

mutant nucleotide, a restriction fragment length polymorphism (RFLP), a nucleotide deletion, a nucleotide substitution, or any other mammalian nucleic acid sequence of interest in such tissue specimens. As used herein the term "mutant or mutated" as applied to a target neoplastic nucleotide sequence shall be understood to encompass a mutation, a restriction fragment length polymorphism, a nucleic acid deletion, or a nucleic acid substitution.

In one embodiment, the method of the invention is applicable to detection of mutant nucleotide sequences associated with benign as well as malignant neoplasias and tumors. In a preferred embodiment, neoplasia of the head or neck, is detected, although the method can be used to detect any neoplastic mutant nucleotide sequence, regardless of origin, as long as the sequence is detectably present in a histologic specimen. For example, neoplasia of regional lymph nodes associated with a primary mammary tumor can be detected utilizing the method of the invention. The specimen can also be chyle or blood.

Numerous nucleic acids having mutant nucleotide sequences that produce an abnormal gene product are known to be associated with various neoplasias.
Among the most common mutant nucleotide sequences are those occurring in oncogenes and tumor suppressor genes, such as mutations of p53 and K-ras. Of special significance in the present invention is the detection of mutations of the p53 tumor suppressor gene (Vogelstein, *Nature*, 348:681, 1990).

Nearly 100 oncogenes have been identified. Though the number of known tumor suppressor genes is far less, the number is growing rapidly. Some of the known or candidate tumor suppressor genes and the neoplasias with which they are associated (J. Marx, *Science*, 261:1385-1367, 1993) are shown in Table 1 below.